Hazard and Risk Assessment of Chemical Mixtures Using the Toxic Equivalency Factor Approach

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There is considerable public, regulatory, and scientific concern regarding human exposure to endocrine-disrupting chemicals, which include compounds that directly modulate steroid hormone receptor pathways (estrogens, antiestrogens, androgens, antiandrogens) and aryl hydrocarbon receptor (AhR) agonists, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Based on quantitative structure–activity relationships for both AhR and estrogen receptor (ER) agonists, the relative potency (RP) of individual compounds relative to a standard (e.g., TCDD and 17β-estradiol) have been determined for several receptor-mediated responses. Therefore, the TCDD or estrogenic equivalent (TEQ or EQ, respectively) of a mixture is defined as $TEQ = \Sigma[T_i] \times RP_i$ or $EQ = \Sigma[E_i] \times RP_i$, where T_i and E_i are concentrations of individual AhR or ER agonists in any mixture. This approach for risk assessment of endocrine-disrupting mixtures assumes that for each endocrine response pathway, the effects of individual compounds are essentially additive. This paper will critically examine the utility of the TEQ/EQ approach for risk assessment, the validity of the assumptions used for this approach, and the problems associated with comparing low dose exposures to xeno and natural (dietary) endocrine disruptors. — *Environ Health Perspect* 106(Suppl 4):1051–1058 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/1051-1058safe/abstract.html*

Key words: endocrine disruptors, TCDD, TEFs, hazard, limitations, interactions

Introduction

The potential adverse impacts of chemicals are dependent on a number of factors, including levels and duration of exposure, chemical potency, timing of exposure, mechanism of action, and interactions between chemicals in a mixture. Hazard and risk assessment of chemicals carried out by regulatory agencies have focused primarily on the toxicities of individual compounds, whereas wildlife and humans are exposed to complex mixtures of man-made compounds that act through multiple pathways. Moreover, the human diet contains many natural products and cooking-derived compounds that exhibit many of the same toxic, mutagenic, and carcinogenic properties of industrial-derived contaminants (*1–3*). In most cases, humans are exposed to significantly higher levels of natural products than the man-made chemical toxicants that act through the same pathway. For example, early development of the Ames test for detecting bacterial mutagens generated considerable scientific, regulatory, and public concern over human exposure to the many different industrial chemicals that exhibited mutagenic activity in one or more of the highly sensitive bacterial tester strains (*4,5*). Subsequent studies demonstrated that some of the most mutagenic compounds in the human diet are not industrial-derived contaminants, but natural compounds that include a complex series of heterocyclic aromatic amines derived from cooking proteinaceous foods (e.g., fish, beef, poultry) (*6–8*). Thus, the public health concern regarding human exposure to mutagens must take into account intake and potency of both natural and man-made chemicals that act through various pathways.

Hazard and risk assessment of human exposures to chemicals must also take into account scenarios where chemical interactions may significantly influence toxic outcomes. For example, despite relatively high levels of human exposure to natural carcinogens in the diet, there are several other classes of natural products (e.g., flavones, antioxidants) that inhibit P450-mediated metabolic activation or induce detoxifying enzymes, and these compounds may provide protection against natural or man-made toxins (*9–11*). In contrast, workplace or environmental exposures to nontoxic levels of organochlorine solvents such as chloroform may lead to hepatotoxic effects if there is concurrent exposure to ketones because of their nonadditive (synergistic) interactions (*12,13*). Thus, chemical interactions are important determinants in evaluating the potential hazards and risks of exposure to chemical mixtures. This manuscript will outline the development, validation, and pitfalls associated with the toxic equivalency factor (TEF) approach for risk assessment of complex mixtures.

Toxic Equivalency Factors: An Approach for Hazard and Risk Assessment

The TEF approach has been extensively used for hazard assessment of different classes of toxic chemical mixtures. The overall toxicity or toxic equivalents (TEQs) of a mixture are defined by the concentration of individual compounds (C_i) in a mixture times their relative potencies or TEFs.

$$TEQ = \Sigma[C_i] \times TEF_i$$

The assumptions implicit in utilization of the TEF approach include: the individual compounds all act through the same biologic or toxic pathway; the effects of individual chemicals in a mixture are essentially additive at submaximal levels of exposure; the dose–response curves for different congeners should be parallel; and the organotropic manifestations of all congeners must be identical over the relevant range of doses (*14,15*). TEF_i values are either derived for a

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Abbreviations used: AhR, aryl hydrocarbon receptor; B[*a*]P, benzo[*a*]pyrene; CB, chlorobiphenyl; E_2, 17β-estradiol; EQ, estrogen equivalents; ER, estrogen receptor; HAH, halogenated aromatic hydrocarbon; MGP, manufactured gas plant; PAH, polynuclear aromatic hydrocarbon; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PCN, polychlorinated naphthalene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEF, toxic equivalency factor; TEQ, toxic equivalent.

species-specific response or are a composite value obtained from TEFs for several responses, and individual TEFs are usually determined relative to the activity of a standard or reference compound. The TEF approach has been applied to different structural classes of compounds, including polynuclear aromatic hydrocarbons (PAHs), halogenated aromatic hydrocarbons (HAHs), and endocrine disruptors. The utility and problems associated with TEFs and TEQs will be discussed.

Toxic Equivalency Factor Approach for Polynuclear Aromatic Hydrocarbons

Individual PAHs such as benzo[*a*]pyrene (B[*a*]P) have been extensively investigated as carcinogens and as ligands for the aryl hydrocarbon receptor (AhR). The carcinogenic activity of PAHs is dependent on the oxidative metabolic activation of these compounds into genotoxic metabolites, which subsequently interact with DNA to initiate carcinogenesis. The carcinogenic potencies of individual PAHs have been determined in different bioassays and TEF values proposed for various PAHs are summarized in Table 1 (*16–19*). The utility of this approach was demonstrated in studies using relatively simple reconstituted PAH mixtures in rodent carcinogenicity models (*20,21*). However, Warshawsky and coworkers (*22*) recently demonstrated that there are a number of important factors that can significantly modulate the genotoxicity of PAH mixtures, including the presence or absence of B[*a*]P, the dose, and the solvents used in carcinogen administration. This variability of carcinogenic potency suggests that the TEF approach for PAHs may not be appropriate for some animal models. Studies in several laboratories have investigated the biochemical, toxic, and genotoxic activities of manufactured gas plant (MGP) residues, which contain complex mixtures of PAHs (*23–27*). Comparisons of the effects of MGP PAHs with B[*a*]P or a reconstituted mixture of PAH hydrocarbons suggested that the mixture induced synergistic responses or that other factors were important. Based on results of recent studies (*24,25,28*), the high activity/genotoxicity of the MGP–PAH mixture may be due to unidentified alkyl PAHs. For example, a reconstituted mixture of the 17 major PAHs in an MGP sample (*24*) did not induce liver tumor formation in the B6C3F$_1$ male juvenile mouse model at a dose of 1071 mg/kg; in contrast, the field-derived sample induced a 45% incidence of liver tumors at the same dose (*25,28*). These results demonstrate that applications of TEFs for PAHs require a more detailed knowledge of the complete composition of these mixtures and the TEFs of all active components. Thus, the approach may be useful for defined PAH mixtures containing only parent hydrocarbons; however, for mixtures containing alkyl PAHs, the TEF approach is not valid because of the minimal data available on the identities and relative potencies of these compounds.

Toxic Equivalency Factor Approach for Halogenated Aromatic Hydrocarbons

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated naphthalenes (PCNs), and polychlorinated biphenyls (PCBs) are HAHs that are industrial compounds or industrial combustion by-products (Figure 1). These compounds are chemically and environmentally stable and have been identified in almost every component of global ecosystems, including fish and wildlife and human serum, adipose tissue, and milk (*29,30*). HAHs are also routinely detected as residues in diverse food products, and the diet is the major source of human exposure to HAHs (*30,32*).

The mechanism of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related HAHs has been extensively investigated and the results support a pathway that involves initial ligand (HAH) binding to the intracellular AhR, which is widely expressed in mammalian tissues (*33*). The mechanism of AhR-mediated CYP1A1 induction has been extensively investigated; the results show that the AhR is a ligand-induced nuclear transcription factor in which transactivation is associated with interaction of the heterodimeric nuclear AhR complex with dioxin-responsive elements located in the 5′-promoter region of the Ah-responsive gene (*34,35*). The mechanisms of AhR-mediated toxicity are

Table 1. Different toxic equivalency factor values proposed for individual polycyclic aromatic hydrocarbon congeners.

Compound	Thorslund et al. (*18*)	Chu and Chen (*17*)	U.S. EPA (*19*)	Nisbet and LaGoy (*16*)
Benzo[*a*]pyrene	1	1	1	1
Dibenzo[*a,h*]anthracene	1.1	0.69	1	5
Benzo[*a*]anthracene	0.145	0.013	0.1	0.1
Benzo[*b*]fluoranthene	0.140	0.08	0.1	0.1
Benzo[*k*]fluoranthene	0.066	0.004	0.01	0.1
Idendo(1,2,3-*c,d*)pyrene	0.232	0.017	0.1	0.1
Acenaphthene	ND	ND	0	0.001
Acenaphthylene	ND	ND	0	0.001
Anthracene	0.32	ND	0	0.01
Benzo[*g,h,i*]perylene	0.022	ND	0	0.01
Chrysene	0.0044	0.001	0.001	0.01
Fluoranthene	ND	ND	0	0.001
Fluorene	ND	ND	0	0.001
2-Methylnaphthalene	ND	ND	0	0.001
Naphthalene	ND	ND	0	0.001
Phenanthrene	ND	ND	0	0.001
Pyrene	0.081	ND	0	0.001

Abbreviations: ND, not determined; U.S. EPA, U.S. Environmental Protection Agency.

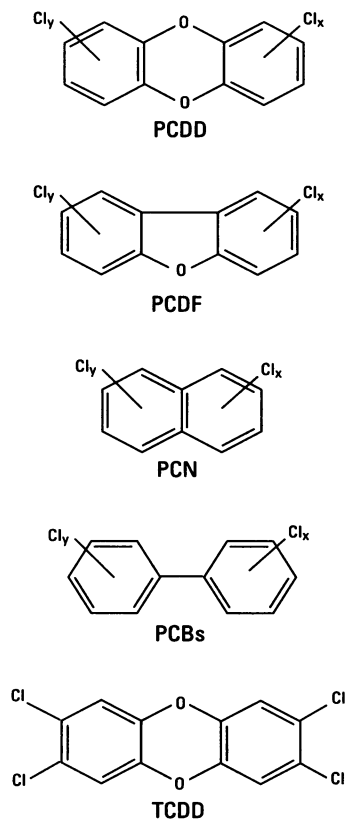

Figure 1. Halogenated aromatic hydrocarbons.

unknown; however, it is assumed that many of the responses are because of altered gene expression.

Hazard and risk assessment of PCDDs and PCDFs initially focused on quantitation of TCDD in various environmental samples; however, with development of high-resolution analytical techniques coupled with studies on structure–toxicity relationships, it was apparent that the bulk of the toxicity induced by most PCDD/PCDF mixtures is not due to TCDD alone. Based on the well-characterized structure–activity relationships established for PCDDs and PCDFs (*36–39*), a TEF approach has been developed for these compounds:

$$TEQ = \Sigma[PCDF_i] \times TEF_i \text{ and } \Sigma[PCDD_i] \times TEF_i$$

where $[PCDD_i]$ and $[PCDF_i]$ represent the concentrations of the individual compounds (*40–46*). Individual TEFs have been assigned to all the 2,3,7,8-substituted PCDD and PCDF congeners (Table 2) because these are the compounds that have primarily been detected in environmental samples and are among the most potent congeners. The TEF for each 2,3,7,8-substituted congener compared to TCDD is variable among cell types, laboratory animal species, target organs, and responses. Research in our laboratory has extensively investigated the immunotoxicity-derived TEFs for several HAHs in mouse models (*47–50*); TEFs for inhibition of the plaque-forming cell response to trinitrophenyl–lipopolysaccharide by 2,3,4,7,8-pentachlorodibenzofuran in C57BL/6, DBA/2, and B6C3F$_1$ mice varied by approximately 7-fold, and in some assays this congener was more potent than TCDD. Over a broader spectrum of responses, TEFs for individual PCDD/PCDF congeners can vary by over 100-fold. The broad range of TEF values for a specific congener compromises the use of a single TEF for this congener and may over- or underestimate the calculated TEQ for a mixture. Variable TEFs are due to many factors including differential pharmacokinetics and metabolism of HAHs in various *in vivo* and *in vitro* bioassays.

Table 2. Toxic equivalency factors for the 2,3,7,8-substituted PCDDs and PCDFs and selected polychlorinated biphenyl congeners.

Congener	TEF
PCDDs	
2,3,7,8-TCDD	1.0
1,2,3,7,8-PentaCDD	0.5
1,2,3,4,7,8-HexaCDD	0.1
1,2,3,6,7,8-HexaCDD	0.1
1,2,3,7,8,9-HexaCDD	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01
OCDD	0.001
PCDFs	
2,3,7,8-TCDF	0.1
2,3,4,7,8-PentaCDF	0.5
1,2,3,7,8-PentaCDF	0.1/0.05
1,2,3,4,7,8-HexaCDF	0.1
2,3,4,6,7,8-HexaCDF	0.1
1,2,3,6,7,8-HexaCDF	0.1
1,2,3,7,8,9-HexaCDF	0.1
1,2,3,4,6,7,8-HeptaCDF	0.01
1,2,3,4,7,8,9-HeptaCDF	0.01
OCDF	0.001
PCBs	
3,3′,4,4′,5-PentaCB	0.1
3,3′,4,4′,5,5′-HexaCB	0.01
3,3′,4,4′-TetraCB	0.0005
2,3,3′,4,4′-PentaCB	0.0001
2,3,3′,4,4′,5-HexaCB	0.0005
2,3′,4,4′,5-PentaCB	0.0001
2,3,3′,4,4′,5′-HexaCB	0.0005
2′,3,4,4′,5-PentaCB	0.0001
2,3,4,4′,5-PentaCB	0.0005

Data from Ahlborg et al. (*45,67*).

Validation of the TEF approach can be investigated by determining the *in vitro* or *in vivo* toxicities of reconstituted mixtures of PCDDs and PCDFs and comparing their observed versus calculated potencies. Eadon and co-workers (*51*) utilized a PCDF/PCDD mixture (primarily PCDFs) resulting from a PCB fire and compared the calculated versus observed effects for several end points in the guinea pig, including decreased body and thymus weights, increased serum triglycerides, decreased serum alanine aminotrasferase levels, and formation of hepatocellular cytoplasmic inclusion bodies. Their results showed that the experimentally observed TEQs per kilogram ranged from 2 to 21 ppm depending on end point; the calculated value using a set of provisional TEFs was 14.5 ppm. These results demonstrated a good correspondence between the observed and calculated values. Other reports using multiple end points in both *in vivo* and *in vitro* models demonstrated that for several PCDD/PCDF mixtures, there is a reasonable correspondence between calculated and experimentally determined TEQs (*52–60*). For more complex mixtures containing compounds that act through multiple pathways to give both similar and different toxic responses, the TEF/TEQ approach may not be appropriate. Moreover, it should also be noted that even for PCDDs/PCDFs, there is some evidence that TEFs do not always predict relative congener potency in different rat strains (*61*).

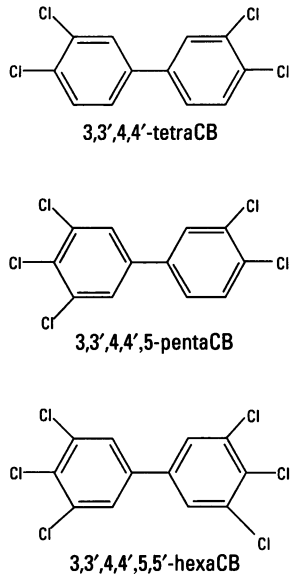

Figure 2. Coplanar (non-*ortho*) polychlorinated biphenyl congeners.

Several studies have also demonstrated that the coplanar PCBs (i.e., 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta, and 3,3′,4,4′,5,5′-hexachlorobiphenyl [CB]) (Figure 2) bind to the AhR and induce a broad spectrum of AhR-mediated biochemical and toxic responses (*62,63*). Tanabe and co-workers (*64,65*) first utilized CYP1A1 induction-derived TEFs (*66*) for coplanar PCBs and their mono-*ortho*-substituted analogs to show that these compounds contributed substantially to the TEQs of diverse industrial/environmental extracts. Provisional TEFs have been proposed for coplanar and mono-*ortho* coplanar PCBs and these values are used routinely for determining total TEQs (i.e., PCDDs, PCDFs, and PCBs) in various extracts (*67*). The relative TEQ contributions of different classes of HAHs are variable; however, there are numerous examples that demonstrate that PCB TEQs contribute > 50% of total TEQs. For example, a recent study on HAHs in a human milk sample (2 weeks after birth) in the Netherlands showed that TEQ values for PCDDs/PCDFs and PCBs were 30.2 and 35.5 ppt (fat weight basis), respectively (*68*).

The major problems associated with the TEF approach for HAHs is primarily associated with nonadditive antagonistic interactions between AhR agonists (PCDDs/PCDFs and PCBs) and PCB congeners that exhibit response and cell/species-specific

Table 3. Examples of antagonistic interactions of halogenated aromatic hydrocarbons: inhibition of TCDD or 3,3′,4,4′,5-pentaCB-induced responses.

Antagonists	Response, animal cell
1,3,6,8-TetraCDF; 2,4,6,8-tetraCDF; Aroclor 1254	AHH/EROD activities, H4IIE cells
2,2′,4,4′,5′,6-HexaCB	EROD activity, chick embryo hepatocytes
2,2′,5,5′-TetraCB	Luciferase activity, mouse and rat cell lines
Aroclors 1232, 1242, 1248, 1254, and 1260; reconstituted PCB mixtures; 1,3,6,8-tetraCDF, 2,3,3′,4,4′,5-HexaCB; 2,3,3′,4,5,5′-hexaCB; 2,3,3′,4,5′-PentaCB; 2,3,4,4′,5,6-hexaCB; 2,2′,4,4′,5,6′-HexaCB; 2,2′,4,4′,6,6′-hexaCB; 2,2′,4,4′,5,5′-HexaCB	Splenic plaque-forming cell response to sheep red blood cells or trinitrophenyl–lipopolysaccharide, mouse strains
2,2′,4,4′,5,5′-hexaCB	Serum IgM units, mice
Aroclor 1254; 2,2′,4,4′,5,5′-hexaCB; 2,2′,5,5′-tetraCB	Fetal cleft palate and hydronephrosis, mice
2,2′,4,4′,5,5′-HexaCB	Chick embryotoxicity, malformations, edema, liver lesions

Abbreviations: AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase. Data from multiple studies (*69–84*).

antagonistic activity (*69–84*) (Table 3). Research in our laboratory has clearly demonstrated that several PCB congeners and some commercial mixtures exhibit AhR antagonist activity. For example, 2,2′,4,4′,5,5′-hexaCB (PCB 153), a major persistent congener in environmental samples, inhibits the following TCDD or 3,3′,4,4′,5-pentachlorobiphenyl-induced responses: induction of ethoxyresorufin *O*-deethylase activity in chick embryo hepatocytes; inhibition of the plaque-forming cell response to sheep red blood cells in mice; inhibition of the plaque-forming cell response to trinitrophenyl lipopolysaccharide in mice; inhibition of serum IgM units in mice; induction of fetal cleft palate in mice; induction of chick embryo malformations; induction of chick embryo edema; induction of chick embryo liver lesions; and induction of fetal hydronephrosis in mice. These nonadditive interactions, coupled with the unusually broad range of TEF values observed for some PCB congeners (e.g., 3,3′,4,4′-tetraCB), compromises the utility of the TEF approach for hazard and risk assessment of HAHs that contain PCBs. Therefore, TEFs/TEQs for HAHs must be used very selectively, and more research on the utility, applications, and limitations of this method should be conducted.

Toxic Equivalency Factor Approach for Endocrine Disruptors

It has been hypothesized that industrial-derived estrogenic compounds (xenoestrogens) and possibly other naturally occurring estrogens may be responsible for a global decrease in male reproductive capacity (e.g., sperm counts) and increased incidence of breast cancer in women (*85,86*). The validity of these hypotheses has been questioned (*1,87*), and resolution of the role of hormonally active compounds in human disease requires further study.

Like AhR agonists, hormonelike compounds that act through specific cellular receptors should be good candidates for using a TEF approach. Verdeal and Ryan (*88*) previously compared human exposures to man-made and naturally occurring estrogenic compounds using a TEF/TEQ approach and diethylstilbestrol equivalents. The recent discovery of a second form of the estrogen receptor (ER), ER_β (*89*), further complicates development of an estrogen equivalent (EQ) approach for estrogenic compounds. The specific responses that are mediated via ER_α or ER_β have not been delineated, and relative potency factors for these responses by different structural classes of natural and man-made estrogenic compounds have not been determined. Several groups have reported TEFs for both natural ligands for the ER (e.g., flavonoids, lignans) and for xenoestrogens, which are industrial-derived chemicals and their by-products (*90–93*). Although individual TEFs have been assigned for each compound, most assay systems indicate that with few exceptions, both natural and xenoestrogens are > 1000 times less potent than 17β-estradiol (E_2). A close inspection of the data obtained for estrogenic compounds reveals that there are many problems in development of a TEF approach for these compounds and some of these problems are similar to those observed for HAHs and PAHs.

Pharmacokinetics, Metabolism, and Serum Protein Binding

The *in vivo* activity of natural and man-made endocrine active agents are significantly influenced by their uptake, distribution, and metabolism. For example, many of the organochlorine xenoestrogens exhibit low estrogenic potency based on results of *in vitro* bioassays; however, these compounds persist in the environment and bioaccumulate. In contrast, many naturally occurring estrogenic flavonoids in foods are rapidly metabolized. For example, studies in this laboratory (*94*) showed that naringenin, a flavonoid in grapefruit juice, exhibited estrogenic activity in *in vitro* bioassays, whereas at doses as high as 30 to 40 mg/animal naringenin, did not induce estrogen-sensitive responses in the rat uterus. In contrast, in female rats cotreated with E_2 plus naringenin, there was significant inhibition of E_2-induced uterine wet weight, progesterone receptor levels, peroxidase activity, and DNA synthesis. Bisphenol A and *p*-octylphenol are two estrogenic phenolic compounds that exhibit similar estrogenic potency in a number of *in vitro* assays (*90*). Vom Saal and co-workers (*95*) recently reported that prenatal to early postnatal exposure of mice to bisphenol A resulted in increased prostate weight in adult male offspring, whereas *p*-octylphenol was inactive in this model. The increased toxicity of bisphenol A compared to *p*-octylphenol was associated with preferential binding of the latter compound to serum proteins and decreased uptake in target cells. In contrast, research in this laboratory in the immature female rat uterus indicated that nonylphenol was significantly more potent than bisphenol A, which exhibited weak ER agonist and partial antiestrogenic activity. Thus, the potencies of both compounds are highly variable and response/species specific, suggesting that a TEF approach would have to incorporate factors that address some measure of response specificity.

Interactions of Endocrine-Active Compounds

Application of a TEF approach assumes additive responses for compounds or mixtures that act through the same pathway at submaximal doses. Arnold and co-workers (*96*) initially reported that binary mixtures of weakly estrogenic organochlorine pesticides, including dieldrin, chlordane, toxaphene, and endosulfan, exhibited > 90-fold and > 160- to 1600-fold synergistic ER binding and induction of reporter gene activity in a yeast-based assay, respectively,

compared to that observed for the compounds alone. This type of nonadditive interaction would negate a TEF/EQ approach for hazard assessment of these mixtures. However, studies from other laboratories (*97,98*) using the same compounds have not observed synergism. The paper on synergism was recently withdrawn (*99*); however, this does not preclude the possibility of other synergistic interactions.

Interactions between Endocrine Response Pathways

The TEF/EQ approach is most applicable for hazard and risk assessment of a specific class of endocrine active compounds that act through a common receptor. However, as noted previously, there are a number of factors that complicate this approach and the problems are magnified with xenoendocrine active agents that act through steroid hormone receptor or thyroid hormone receptor-mediated pathways. For example, assessment of xenoestrogen exposure and potency (EQs) is complicated by tissue-specific agonist/antagonist activities, lack of data on intake and serum levels, and their relative contribution to total estrogen equivalents compared to much higher intakes of natural estrogenic chemicals in the diet (*1*). In addition, many compounds may interact with more than one hormone receptor and modulate multiple endocrine response pathways. For example, 2′,3′,4′,5′-tetrachloro-2-biphenylol binds to the ER and exhibits ER-agonist activities (*100,101*). The same compound also binds to the androgen receptor in a yeast-based assay but inhibits dihydrotestosterone-induced reporter gene activity in human Hep G2 liver cancer cells transiently transfected with the human androgen receptor and an androgen-responsive construct (*102*). Although 2′,3′,4′,5′-tetrachloro-4-biphenylol did not bind the progesterone receptor, in a progesterone-responsive yeast assay this hydroxy-PCB inhibited progesterone receptor-mediated transactivation (*103*). These data illustrate how one endocrine-active compound can modulate multiple endocrine response pathways.

Another major problem for hazard assessment of xenoestrogens is associated with tissue-specific cross talk between different receptor-mediated pathways, which can lead to significant modulation of estrogen-induced responses. It has been pointed out that in human breast cancer cells, cross talk between the ER- and AhR-signaling pathway results in inhibition of estrogen-induced responses (*1*). Although this interaction is likely to be cell specific, it is possible that the estrogenic activity associated with xenoestrogens in the mammary gland will be inhibited by both xeno AhR and natural AhR agonists in the diet. Research in this laboratory has also focused on cross talk between the ER and other receptors that bind natural dietary constituents. Vitamin A, retinoids, phytol, and phytanic acid are vitamins or plant degradation products and are also important dietary constituents that act through the retinoic acid and retinoic acid X receptors, and there is cross talk between these receptors and the ER. For example, all *trans*-retinoic acid, 9-*cis*-retinoic acid, and phytol inhibit estrogen-induced responses in breast cancer cells, and any effects of xenoestrogens in these cells would be opposed by retinoic acid and retinoic acid X receptor ligands. The importance of this type of counteractive cross talk in other tissues and organs has not been determined. These are only some examples of cross talk between endocrine-signaling pathways that must be considered in an overall risk assessment of dietary exposure to xenoestrogens and other xenoendocrine active compounds, as well as natural compounds in food that act through the same signaling pathways.

Summary

Humans and wildlife are exposed in the diet to complex mixtures of natural and man-made chemicals. Hazard and risk assessment of these mixtures is a difficult process and the TEF approach has been utilized for several different classes of chemicals, including HAHs (AhR agonists), PAHs (carcinogens), and xenoestrogens (ER agonists). This review has pointed out both the utility and problems associated with the TEF approach for all three classes of chemicals. For example, although TEFs may be useful for regulating HAH emissions and cleanup levels, application of this concept for determining dietary TEQ intakes is complicated by the unknown contributions of naturally occurring AhR agonists, which exhibit both AhR agonist and antagonist activities (*2*). Moreover, the issue of cross talk between multiple endocrine pathways would further compromise the validity of the TEF approach when applied to dietary intakes of different classes of man-made and natural chemicals. Based on these uncertainties, the TEF approach should be used for limited applications and only after validation in animal models.

REFERENCES AND NOTES

1. Safe S. Environmental and dietary estrogens and human health: is there a problem? Environ Health Perspect 103:346–351 (1995).
2. Safe S. Development, validation and problems with the TEF approach for risk assessment of dioxins and related compounds. J Anim Sci 76:134–141 (1998).
3. Silkworth JB, Brown JF Jr. Evaluating the impact of exposure to environmental contaminants on human health. Clin Chem 42:1345–1349 (1996).
4. Ames BN, Durston WE, Yamasaki E, Lee FD. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285 (1973).
5. McCann J, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc Natl Acad Sci USA 72:5135–5139 (1975).
6. Nagao M, Ushijima T, Wakabayashi K, Ochiai M, Kushida H, Sugimura T, Hasegawa R, Shirai T, Ito N. Dietary carcinogens and mammary carcinogenesis. Induction of rat mammary carcinomas by administration of heterocyclic amines in cooked foods. Cancer 74:1063–1069 (1994).
7. Ames BN. Mutagenesis and carcinogenesis: endogenous and exogenous factors. Environ Mol Mutagen 14(Suppl 16):66–77 (1989).
8. Ames BN, Profet M, Gold LS. Nature's chemicals and synthetic chemicals: comparative toxicology. Proc Natl Acad Sci USA 87:7782–7786 (1990).
9. Talalay P. Mechanisms of induction of enzymes that protect against chemical carcinogenesis. Adv Enzyme Regul 28:237–250 (1989).
10. Barch DH, Rundhaugen LM, Pillay NS. Ellagic acid induces transcription of the rat glutathione *S*-transferase-Ya gene. Carcinogenesis 16:665–668 (1995).
11. Siess MH, Leclerc J, Canivenc-Lavier MC, Rat P, Suschetet M. Heterogenous effects of natural flavonoids on monooxygenase activities in human and rat liver microsomes. Toxicol Appl Pharmacol 130:73–78 (1995).

12. Hewitt WR, Brown EM, Plaa GL. Relationship between the carbon skeleton length of ketonic solvents and potentiation of chloroform-induced hepatotoxicity in rats. Toxicol Lett 16:297–304 (1983).
13. Cowlen MS, Hewitt WR, Schroeder F. Mechanisms in 2-hexanone potentiation of chloroform hepatotoxicity. Toxicol Lett 22:293–299 (1984).
14. NATO/CCMS. Scientific Basis for the Development of International Toxicity Equivalency Factor (I-TEF), Method of Risk Assessment for Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Washington:North Atlantic Treaty Organization/Committee on the Challenges of Modern Society, 1988;178.
15. Nagao T, Golor G, Hagenmaier H, Neubert D. Teratogenic potency of 2,3,4,7,8-pentachlorodibenzofuran and of three mixtures of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mice. Problems with risk assessment using TCDD toxic-equivalency factors. Toxicology 57:591–597 (1993).
16. Nisbet ICT, LaGoy PK. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regul Toxicol Pharmacol 16:290–300 (1992).
17. Chu MML, Chen CW. Evaluation and estimation of potential carcinogenic risks of polynuclear aromatic hydrocarbons. Presented at the Symposium on Polycyclic Aromatic Hydrocarbons in the Workplace 1984, Honolulu, HI.
18. Thorslund TW, Charnley G, Anderson EL. Unpublished data.
19. U.S. EPA. Health Effects Assessment of Polycyclic Aromatic Hydrocarbons (PAHs). EPA 540/1-86-013. Cincinnati, OH: U.S. Environmental Protection Agency Environmental Criteria and Assessment Office, 1984.
20. Pfeiffer EH. Oncogenic interaction of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons. In: Air Pollution and Cancer in Man. IARC Sci Publ 16 (Mohr U, Schmähl D, Tomatis L, eds). Lyon:International Agency for Cancer Research, 1977;67–77.
21. Schmahl D, Schmidt KG, Habs M. Syncarcinogenic action of polycyclic aromatic hydrocarbons in automobile exhaust gas condensates. In: Air Pollution and Cancer in Man. IARC Sci Publ 16 (Mohr U, Schmähl D, Tomatis L, eds). Lyon:International Agency for Cancer Research, 1977;53–59.
22. Warshawsky D, Barkley W, Bingham E. Factors affecting carcinogenic potential of mixtures. Fundam Appl Toxicol 20:376–382 (1993).
23. Chaloupka K, Harper N, Krishnan V, Santostefano M, Rodriguez LV, Safe S. Synergistic activity of polynuclear aromatic hydrocarbon mixtures as aryl hydrocarbon (Ah) receptor agonists. Chem Biol Interact 89:141–158 (1993).
24. Chaloupka K, Steinberg M, Santostefano M, Rodriguez LV, Goldstein L, Safe S. Induction of *Cyp1a-1* and *Cyp1a-2* gene expression by a reconstituted mixture of polynuclear aromatic hydrocarbons in $B6C3F_1$ mice. Chem Biol Interact 96:207–221 (1995).
25. Rodriguez LV, Dunsford HA, Steinberg M, Chaloupka K, Zhu L, Safe S, Womack JE, Goldstein LS. Carcinogenicity of benzo[*a*]pyrene and manufactured gas plant residues in infant mice. Carcinogenesis 18:127–135 (1997).
26. Weyand EH, Wu Y. Covalent binding of polycyclic aromatic hydrocarbon components of manufactured gas plant residue to mouse lung and forestomach DNA. Chem Res Toxicol 8:955–962 (1995).
27. Weyand EH, Chen YC, Wu Y, Koganti A, Dunsford HA, Rodriguez LV. Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[*a*]pyrene vs manufactured gas plant residue. Chem Res Toxicol 8:949–954 (1995).
28. Steinberg MA. Role of Polycyclic Aromatic Hydrocarbons in Coal Tar-Induced Genotoxicity and Carcinogenicity. PhD Thesis. College Station, TX:Texas A&M University, 1996.
29. McFarland VA, Clarke JU. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ Health Perspect 81:225–239 (1989).
30. U.S. EPA. Health Assessment for 2,3,7,8-TCDD and Related Compounds. External Review Draft. EPA/600/BP-92/001a-c. Washington:U.S. Environmental Protection Agency, 1994.
31. Tanabe S, Iwata H, Tatsukawa R. Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. Sci Total Environ 154:163–177 (1994).
32. Schecter A, Furst P, Furst C, Papke O, Ball M, Ryan JJ, Hoang DC, Le CD, Hoang TQ, Cuong HQ. Chlorinated dioxins and dibenzofurans in human tissue from general populations: a selective review. Environ Health Perspect 102(Suppl 1):159–171 (1994).
33. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons. Examinations of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 22:517–554 (1982).
34. Whitlock JP Jr. Mechanistic aspects of dioxin action. Chem Res Toxicol 6:754–763 (1993).
35. Safe S. Modulation of gene expression and endocrine response pathways by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. Pharmacol Ther 67:247–281 (1995).
36. Goldstein JA, Safe S. Mechanism of action and structure-activity relationships for the chlorinated dibenzo-*p*-dioxins and related compounds. In: Halogenated Biphenyls, Naphthalenes, Dibenzodioxins and Related Compounds (Kimbrough RD, Jensen AA, eds). Amsterdam:Elsevier-North Holland, 1989;239–293.
37. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). CRC Crit Rev Toxicol 21:51–88 (1990).
38. Safe S. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Annu Rev Pharmacol Toxicol 26:371–399 (1986).
39. Safe S, Mason G, Sawyer T, Zacharewski T, Harris M, Yao C, Keyes B, Farrell K, Holcomb M, Davis D, et al. Development and validation of *in vitro* induction assays for toxic halogenated mixtures. Toxicol Ind Health 5:757–775 (1989).
40. NATO/CCMS Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Rpt No 176. Washington:NATO/CCMS, 1988.
41. North Atlantic Treaty Organization. Scientific Basis for the Development of International Toxicity Equivalency Factor (I-TEF), Method of Risk Assessment for Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Rpt No 178. Washington:NATO/CCMS, 1988.
42. Ahlborg UG, Håkansson H, Wærn F, Hanberg A. Nordisk dioxinriskbedömning. Rapport fran en nordisk expertgrupp. Miljorapport:Nordisk Ministerrådet (1988).
43. Bellin JS, Barnes DG. Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-dioxins and -dibenzofurans (CDDs and CDFs). Washington:U.S. Environmental Protection Agency, 1989.
44. Barnes DG, Kutz FW, Bottimore DP. Interim Procedures for Estimating Risks Associated with Exposure to Mixtures of Chlorinated Dibenzo-*p*-dioxins and -dibenzofurans and 1989 Update. EPA/625/3-89/016. Springfield, VA:National Technical Information Service, 1989.
45. Ahlborg UG, Brouwer A, Fingerhut MA, Jacobson JL, Jacobson SW, Kennedy SW, Kettrup AAF, Koeman JH, Poiger H, Rappe C, et al. Impact of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls on human and environmental health with special emphasis on application of the toxic equivalence factor concept. Eur J Pharmacol 228:179–199 (1992).
46. Birnbaum LS, DeVito MJ. Use of toxic equivalency factors for risk assessment for dioxins and related compounds. Toxicology 105:391–401 (1995).
47. Davis D, Safe S. Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationships and interactive effects. Toxicol Appl Pharmacol 94:141–149 (1988).

48. Harper N, Connor K, Safe S. Immunotoxic potencies of polychlorinated biphenyl (PCB), dibenzofuran (PCDF) and dibenzo-*p*-dioxin (PCDD) congeners in C57BL/6 and DBA/2 mice. Toxicology 80:217–227 (1993).
49. Harper N, Connor K, Steinberg M, Safe S. An ELISA specific for antibodies to TNP-LPS detects alterations in serum immunoglobulins and isotype switching in C57BL/6 and DBA/2 mice exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. Toxicology 92:155–167 (1994).
50. Harper N, Steinberg M, Thomsen J, Safe S. Halogenated aromatic hydrocarbon-induced suppression of the plaque-forming cell response in $B6C3F_1$ splenocytes cultured with allogenic mouse serum: Ah receptor structure-activity relationships. Toxicology 99:199–206 (1995).
51. Eadon G, Kaminsky L, Silkworth J, Aldous K, Hilker D, O'Keefe P, Smith R, Gierthy JF, Hawley J, Kim N, et al. Calculation of 2,3,7,8-TCDD equivalent concentrations of complex environmental contaminant mixtures. Environ Health Perspect 70:221–227 (1986).
52. Stahl BU, Kettrup A, Rozman K. Comparative toxicity of four chlorinated dibenzo-*p*-dioxins (CDDs) and their mixture. Part I: Acute toxicity and toxic equivalency factors (TEFs). Arch Toxicol 66:471–477 (1992).
53. Sawyer TW, Vatcher AD, Safe S. Comparative aryl hydrocarbon hydroxylase induction activities of commercial PCBs in Wistar rats and rat hepatoma H-4-II E cells in culture. Chemosphere 13:695–701 (1984).
54. Schrenk D, Lipp HP, Wiesmuller T, Hagenmaier H, Bock KW. Assessment of biological activities of mixtures of polychlorinated dibenzo-*p*-dioxins: comparison between defined mixtures and their constituents. Arch Toxicol 65:114–118 (1991).
55. Brown MM, Schneider UA, Petrulis JR, Bunce NJ. Additive binding of polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to the murine hepatic Ah receptor. Toxicol Appl Pharmacol 129:243–251 (1994).
56. Schmitz HJ, Hagenmaier A, Hagenmaier HP, Bock KW, Schrenk D. Potency of mixtures of polychlorinated biphenyls as inducers of dioxin receptor-regulated CYP1A activity in rat hepatocytes and H4IIE cells. Toxicology 99:47–54 (1995).
57. Gierthy JF, Crane D, Frenkel GD. Application of an *in vitro* keratinization assay to extracts of soot from a fire in a polychlorinated biphenyl-containing transformer. Fundam Appl Toxicol 4:1036–1041 (1984).
58. Silkworth J, McMartin D, DeCaprio A, Rej R, O'Keefe P, Kaminsky L. Acute toxicity in guinea pigs and rabbits of soot from a polychlorinated biphenyl-containing transformer fire. Toxicol Appl Pharmacol 65:425–439 (1982).
59. Silkworth JB, Cutler DS, Sack G. Immunotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a complex environmental mixture from the Love Canal. Fundam Appl Toxicol 12:303–312 (1989).
60. Silkworth JB, Cutler DS, Antrim L, Houston D, Tumasonis C, Kaminsky LS. Teratology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a complex environmental mixture from the Love Canal. Fundam Appl Toxicol 13:1–15 (1989).
61. Pohjanvirta R, Unkila M, Lindén J, Tuomisto JT, Tuomisto J. Toxic equivalency factors do not predict the acute toxicities of dioxins in rats. Eur J Pharmacol 293:341–353 (1995).
62. Safe S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action. CRC Crit Rev Toxicol 12:319–395 (1984).
63. Safe S. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit Rev Toxicol 24:87–149 (1994).
64. Kannan N, Tanabe S, Tatsukawa R. Potentially hazardous residues of non-*ortho* chlorine substituted coplanar PCBs in human adipose tissue. Arch Environ Health 43:11–14 (1988).
65. Kannan N, Tanabe S, Tatsukawa R. Toxic potential of non-*ortho* and mono-*ortho* coplanar PCBs in commercial PCB preparations: 2,3,7,8-T_4CDD toxicity equivalence factors approach. Bull Environ Contam Toxicol 41:267–276 (1988).
66. Sawyer T, Safe S. PCB isomers and congeners: induction of aryl hydrocarbon hydroxylase and ethoxyresorufin *O*-deethylase enzyme activities in rat hepatoma cells. Toxicol Lett 13:87–94 (1982).
67. Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, et al. Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28:1049–1067 (1994).
68. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, van der Paauw CG, Tuinstra LGMT, Boersma ER, Sauer PJJ. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 28:1721–1732 (1994).
69. Bannister R, Davis D, Zacharewski T, Tizard I, Safe S. Aroclor 1254 as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist: effects on enzyme activity and immunotoxicity. Toxicology 46:29–42 (1987).
70. Haake JM, Safe S, Mayura K, Phillips TD. Aroclor 1254 as an antagonist of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Lett 38:299–306 (1987).
71. Biegel L, Harris M, Davis D, Rosengren R, Safe L, Safe S. 2,2′,4,4′,5,5′-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6J mice. Toxicol Appl Pharmacol 97:561–571 (1989).
72. Davis D, Safe S. Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Lett 48:35–43 (1989).
73. Biegel L, Howie L, Safe S. Polychlorinated biphenyl (PCB) congeners as 2,3,7,8-TCDD antagonists-teratogenicity studies. Proceedings Dioxin '88 meeting, Umea, Sweden. Chemosphere 19:955–958 (1989).
74. Davis D, Safe S. Immunosuppressive activities of polychlorinated biphenyls in C57BL/6 mice: structure-activity relationships as Ah receptor agonists and partial antagonists. Toxicology 63:97–111 (1990).
75. Morrissey RE, Harris MW, Diliberto JJ, Birnbaum LS. Limited PCB antagonism of TCDD-induced malformations in mice. Toxicol Lett 60:19–25 (1992).
76. Harper N, Connor K, Steinberg M, Safe S. Immunosuppressive activity of polychlorinated biphenyl mixtures and congeners: non-additive (antagonistic) interactions. Fundam Appl Toxicol 27:131–139 (1995).
77. Zhao F, Mayura K, Kocurek N, Edwards JF, Kubena LF, Safe SH, Phillips TD. Inhibition of 3,3′,4,4′,5-pentachlorobiphenyl-induced chicken embryotoxicity by 2,2′,4,4′,5,5′-hexachlorobiphenyl. Fundam Appl Toxicol 35:1–8 (1997).
78. Zhao F, Mayura K, Harper N, Safe SH, Phillips TD. Inhibition of pentachlorobiphenyl-induced fetal cleft palate and immunotoxicity in C57BL/6 mice by 2,2′,4,4′,5,5′-hexachlorobiphenyl. Chemosphere 34:1605–1613 (1997).
79. Tysklind M, Bosveld ATC, Andersson P, Verhallen E, Sinnige T, Seinen W, Rappe C, Van den Berg M. Inhibition of ethoxyresorufin-*O*-deethylase (EROD) activity in mixtures of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polychlorinated biphenyls. Environ Sci Pollut Res 4:211–216 (1995).
80. Davis D, Safe S. Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationships and interactive effects. Toxicol Appl Pharmacol 94:141–149 (1988).
81. Harris GE, Metcalfe TL, Metcalfe CD, Huestis SY. Embryotoxicity of extracts from Lake Ontario rainbow trout (*Oncorhynchus mykiss*) to Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 13:1393–1403 (1995).
82. Keys B, Piskorska-Pliszczynska J, Safe S. Polychlorinated dibenzofurans as 2,3,7,8-TCDD antagonists: *in vitro* inhibition of monooxygenase enzyme induction. Toxicol Lett 31:151–158 (1986).
83. Bosveld ATC, Verhallen E, Seinen W, Van den Berg M. Mixture interactions in the *in vitro* CYP1A1 induction bioassay using chicken embryo heptocytes. Organohalogen Compounds 25:309–312 (1995).

84. Aarts JMMJG, Denison MS, Cox MA, Schalk MAC, Garrison PM, Tullis K, De Haan LHJ, Brouwer A. Species-specific antagonism of Ah receptor action by 2,2′,5,5′-tetrachloro- and 2,2′,3,3′,4,4′-hexachlorobiphenyl. Eur J Pharmacol 293:463–474 (1995).
85. Sharpe RM, Skakkebaek NF. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract. Lancet 341:1392–1395 (1993).
86. Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ Health Perspect 101:372–377 (1993).
87. Ahlborg UG, Lipworth L, Titusernstoff L, Hsieh CC, Hanberg A, Baron J, Trichopoulos D, Adami HO. Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: an assessment of the biological and epidemiological evidence. Crit Rev Toxicol 25:463–531 (1995).
88. Verdeal K, Ryan DS. Naturally-occurring estrogens in plant foodstuffs: a review. J Food Prot 42:577–583 (1979).
89. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930 (1996).
90. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103:113–122 (1995).
91. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103:582–587 (1995).
92. Gaido KW, Leonard LS, Lovell S, Gould JC, Babaï D, Portier CJ, McDonnell DP. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol 143:205–212 (1997).
93. Klotz DM, Beckman BS, Hill SM, McLachlan JA, Walters MR, Arnold SF. Identification of environmental chemicals with estrogenic activity using a combination of *in vitro* assays. Environ Health Perspect 104:1084–1089 (1996).
94. Ruh MF, Zacharewski T, Connor K, Howell J, Chen I, Safe S. Naringenin: a weakly estrogenic bioflavonoid which exhibits antiestrogenic activity. Biochem Pharmacol 50:1485–1493 (1995).
95. Vom Saal FS, Timms BG, Montano MM, Thayer KA, Nagel SC, Dhar MG, Ganjam VK, Parmigiani S, Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci USA 94:2056–2061 (1997).
96. Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ Jr, McLachlan JA. Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 272:1489–1492 (1996).
97. Ramamoorthy K, Wang F, Chen I-C, Norris JD, McDonnell DP, Gaido KW, Bocchinfuso WP, Korach KS, Safe S. Potency of combined estrogenic pesticides. Science 275:405–406 (1997).
98. Ramamoorthy K, Wang F, Chen I-C, Norris JD, McDonnell DP, Gaido KW, Bocchinfuso WP, Korach KS, Safe S. Estrogenic activity of a dieldrin/toxaphene mixture in the mouse uterus, MCF-7 human breast cancer cells and yeast-based estrogen receptor assays: no apparent synergism. Endocrinology 138:1520–1527 (1997).
99. McLachlan JA. Synergistic effect of environmental estrogens: report withdrawn. Science 227:462–463 (1997).
100. Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol Pharmacol 33:120–126 (1988).
101. Ramamoorthy K, Vyhlidal C, Wang F, Chen I-C, Safe S, McDonnell DP, Leonard LS, Gaido KW. Additive estrogenic activities of a binary mixture of 2′,4′,6′-trichloro- and 2′,3′,4′,5′-tetrachloro-4-biphenylol. Toxicol Appl Pharmacol 147:93–100 (1997).
102. Safe S, Connor K, Ramamoorthy K, Gaido KW, Maness SC. Human exposure to endocrine-active chemicals: hazard assessment problems. Regul Pharmacol Toxicol 26:52–58 (1997).
103. Jin L, Tran DQ, Ide CF, McLachlan JA, Arnold SF. Several synthetic chemicals inhibit progesterone receptor-mediated transactivation in yeast. Biochem Biophys Res Commun 233:139–146 (1997).